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Basic Investigation

Effects of Xuesetong Soft Capsules (血塞通软胶囊) on angiogenesis and VEGF mRNA expression in ischemic myocardium in rats with myocardial infarction

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Abstract

OBJECTIVE: To observe the effects of Xuesetong Soft Capsules (血塞通软胶囊, Notoginseng total saponin) on angiogenesis and vascular endothelial growth factor (VEGF) mRNA expression in ischemic myocardium of rats with myocardial infarction.

METHODS: The left coronary artery of rats was ligated to establish the animal model of acute myocardial infarction. Rats were randomly divided into Xuesetong Soft Capsule, Shexiangbaoxin Pill (positive control), model (negative control) and sham operation groups. After 6 weeks, microvessel count (MVC), microvessel density (MVD) and VEGF mRNA expression in ischemic myocardium were evaluated.

RESULTS: MVC and MVD in the myocardial infarct border area in model, Shexiangbaoxin Pill and Xuesetong Soft Capsule groups significantly increased compared with those of the sham operation group (P<0.05). MVC and MVD in the myocardial infarct border area in Xuesetong Soft Capsule and Shexiangbaoxin Pill groups significantly increased compared with those of the model group (P<0.05). No significant differences between Xuesetong Soft Capsule and Shexiangbaoxin Pill groups were observed (P>0.05). The model group showed significantly higher VEGF mRNA expression than that in the sham operation group (P<0.05). Xuesetong Soft Capsule and Shexiangbaoxin Pill groups showed significantly higher VEGF mRNA expression than that of the model group (P<0.05). No significant difference between Xuesetong Soft Capsule and the Shexiangbaoxin Pill groups was observed (P>0.05).

CONCLUSION: Xuesetong Soft Capsules promote angiogenesis in ischemic myocardium after myocardial infarction and the mechanism may be associated with VEGF mRNA expression.

Key words: Xuesetong Soft Capsules (血塞通软胶囊); Notoginseng total saponin; Myocardial infarction; Angiogenesis; vascular endothelial growth factor

INTRODUCTION

Formation and opening of collateral circulation of the coronary artery can relieve myocardial ischemia, avoid cell necrosis, as well as prevent and delay formation of ischemic myocardial diseases, improve clinical symptoms and prognosis, while angiogenesis promotes establishment of collateral circulation in ischemic border tissues. Traditional Chinese Medicine has some prospective therapeutic effects on coronary heart disease. Studies found that some Chinese patent medicines promote or induce angiogenesis in ischemic myocardium[1-3]. Therefore, actively seeking commonly-used Chinese patent medicines that promote angiogenesis in ischemic myocardium is of practical value for treatment of ischemic heart diseases. The main component of Xuesetong Soft Capsules is notoginseng total saponin extract

ed from the Chinese medicine San Qi (Radia Notoginseng), which is widely applied in clinical practice. Experimental and clinical studies indicate that it has various cardiovascular pharmacologic effects. In the present study, the effects of Xuesetong Soft Capsules (血塞通软胶囊) on angiogenesis and vascular endothelial growth factor (VEGF) mRNA expression in ischemic myocardium of rats with myocardial infarction were observed to assess its action on angiogenesis in ischemic myocardium and to investigate the mechanism.

MATERIALS AND MEDTHODS

Drugs for the Experiment

Xuesetong Soft Capsules (血塞通软胶囊, Notoginseng total saponin) were supplied by Kunming Shenghuo Parmaceutical Co. Ltd (Kunming, Yunnan, China), batch No: 20031125. After the capsules were removed, the notoginseng total saponin powder was dissolved in aseptic distilled water at 40 mg/mL.

Shexiangbaoxin Pills (麝香保心丸) were prepared by Shanghai Hehuang Pharmaceutical Co. Ltd (Shanghai, China), batch No: A040214. Shexiangbaoxin Pills were ground and then dissolved in aseptic distilled water at 3 mg/mL.

Reagents

A mouse anti-rat CD34 monoclonal antibody was purchased from Santa Cruz Biotechnology, Inc, (Santa Cruz, CA, USA).

VEGF in situ hybridization kits and diaminobenzidine (DAB) concentrated chromogenic solution kits were purchased from Wuhan Boster Bioengineering Co, Ltd, (Wuhan, Hubei, China).

Instruments

A respirator for small animals (Jiangxi Teli Anesthesia and Respiration Equipments Company, Nanchang, Jiangxi, China), Microscope (OLYMPUS Optic Co. Ltd, Tokyo, Japan), SPOT II Digital camera with SPOT™ RT Software v3.0 (DIAGNOSTIC INSTRUMENTS Inc. Sterling Heights, MI, USA) and a Metamorph Imaging System Version 4.5 (Universal Imaging Corporation, Ypsilanti, MI, USA).

Animal model establishment and grouping

Male Wistar rats, weighing 200±20 g, were used in this study. Based on a previous study[1], after rats were weighted and anesthetized with an interperitoneal injection of 1% pentobarbital at 50 mg/kg body weight, rats were fixed and the skin on the anterior pectoral region was disinfected and endotracheal intubation was fed through the larynx. The small animal respirator was connected for artificial respiration at 80 times/min with a 0.7 – 0.8 mL tidal volume. Skin between the left third-forth ribs was horizontally incised at about 1.5 cm in length, the muscular layers were separated and the thoracic cavity was opened along the space between the third-fourth ribs. The visual field was widened with a thoracotome, the pericardium was incised, and the left coronary artery at about 1 mm below the part between the arterial cone and left auricle was ligated with 6/0 thread. After the standard lead I of the electrocardiogram showed obvious elevation of the ST segment, the heart was returned to the thoracic cavity and the thoracic wall was immediately sutured layer by layer. Penicillin (4×10^5 U/rat) was intraperitoneally injected for 3 consecutive days. For rats in the sham operation group, the operation procedures were the same as described above without ligation of the coronary artery. After the operation, rats were routinely maintained and the myocardial infarction model was determined according to the ST elevation in the five leads I, avL and V_4 – V_6 on the day of modeling. Rats were randomly divided into Xueshetong Soft Capsule, Shexiangbaoxin Pill and model groups. On day 7 after modeling, rats were grouped again. After the five leads I, avL and V_4 – V_6 all with the Q wave determined from rats of the three groups, eight rats in each group were selected for the following 6 week drug treatment experiment.

Treatment Methods

Within 24 h after modeling, medicines were administrated intragastrically each day at 0.4 g/kg notoginseng total saponin for the Xuesetong Soft Capsule group, 30 mg/kg Shexiangbaoxin Pill for the Shexiangbaoxin Pill group, 10 mL/kg saline for the sham operation group, and 10 mL/kg distilled water for the model group. After 6 weeks, rats were sacrificed.

Indexes of observation

Myocardial tissue including the myocardial infarction region, transitional areas and health area were harvested and fixed in 10% neutral formalin buffer, followed by paraffin embedding and preparation of tissue sections.

Determination of myocardial microvessel count (MVC) and microvessel density (MVD) in the myocardial infarction border area

After immunohistochemical staining of tissue sections with the primary antibody (1:100, prepared in a 1% PBS solution), vascular endothelial cells were stained pale brown. The criteria for assessment of microvessels was based on a published method[5], single endothelial cells or endothelial cell clusters stained pale brown were regarded as one blood vessel. The blood vessels with the lumen consisting of more than eight red cells in size and blood vessels with a thicker muscular layer

were not counted. The myocardial infarction border area was examined under 40 × magnification, and microvessels were counted under 400× magnification. For each section, five visual fields were selected and MVC in each visual field was counted and the mean was regarded as the MVC of the individual rat. Because MVD was expressed with microvessel counts/visual field, MVC of each rat was the MVD of rats.

Detection of VEGF mRNA expression in the myocardial infarction border area

For in situ hybridization staining of tissue slices, pale brown granules in cells was regarded as positive VEGF mRNA expression. The mean gray value of each 100× visual field of the myocardial infarction border area was calculated with a pathological image analysis system, with six visual fields determined randomly for each slice. The mean was taken as the gray value of the rat.

Statistical Analysis

Measurement data were expressed as the mean ± standard deviation ($\bar{x} \pm s$). SPSS 13.0 software (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. If the data were a normal distribution and the variances were regular, single factor analysis of variance (ANOVA, LSD) was carried out. If the data were a normal distribution but the variance was irregular, single factor analysis of variance (ANOVA, Dunnett's C) was carried out.

RESULTS

Comparison of MVC and MVD in the myocardial infarction border area of rats among groups

Newly generated microvessels were observed in these groups (see Figure 1). MVC and MVD in model, Shexiangbaoxin Pill and Xuesetong Capsule groups significantly increased compared with those of the sham operation group (all P<0.05). MVC and MVD in Shexiangbaoxin Pill and Xuesetong Capsule groups significantly increased compared with those of the model group (all P<0.05). No differences between Shexiangbaoxin Pill and Xuesetong Capsule groups were observed (P>0.05). See Table 1.

Table 1 Comparison of MVC and MVD in the myocardial infarction border area among groups ($\bar{x} \pm S$)

Group	n	MVC, MVD
Sham operation	8	42.41±2.31$^{*\blacktriangle}$
Model	8	56.96±10.53$^{\#\blacktriangle}$
Shexiang Baoxin	8	82.69±4.89$^{*\#}$
Xuesetong Capsule	8	82.15±6.92$^{*\#}$

Notes: $^{*}P$<0.05 vs model group, $^{\#}P$<0.05 vs sham operation group, $^{\blacktriangle}P$<0.05 vs Shexiangbaoxin Pills group.

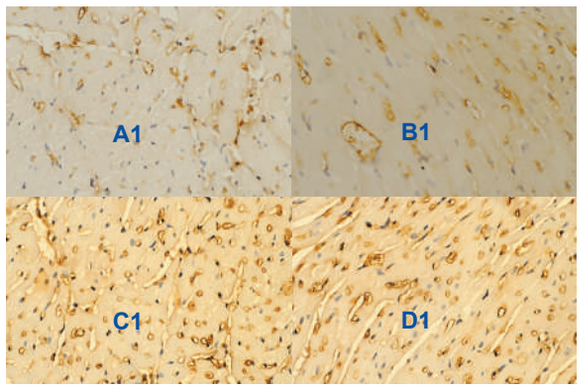

Figure 1 CD34 immunohistochemical staining at the myocardial infarction border. A1: Sham operation group. B1: Model group. C1: Shexiangbaoxin Pills group D1 Xuesetong Soft Capsule group.

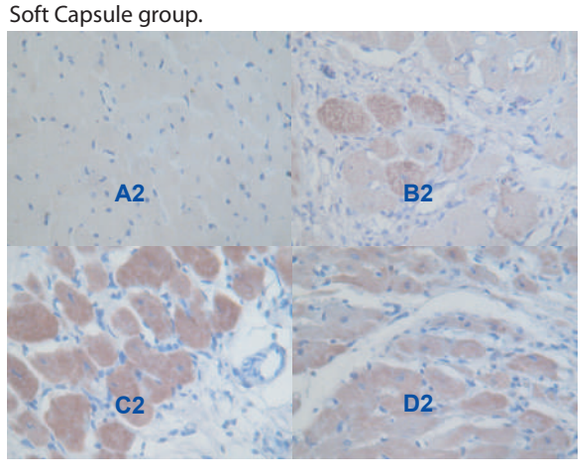

Figure 2 VEGF mRNA expression in the myocardial infarction boader area of rats among groups (×400). A2: Sham operation group. B2: Model group. C2: Shexiangbaoxin Pill group. D2: Xuesetong Soft Capsule group.

VEGF mRNA expression in the myocardial infarction border area of rats among groups

Under a light microscope, positive VEGF mRNA stained pale brown. The positive staining area was distributed mainly in the cytoplasm of myocardial cells. VEGF mRNA expression in the myocardial infarction border area of rats among groups is shown in Figure 2 and Table 2. The results indicated that VEGF mRNA expression in the model group was higher than that in the sham operation group (P<0.05). VEGF mRNA expression in Xueshetong Soft Capsule and Shexiangbaoxin Pill groups were significantly higher than that in the model group (both P<0.05). No significant differences between Xueshetong Soft Capsule and Shexiangbaoxin Pill groups were observed (P>0.05).

Table 2 VEGF mRNA expression in the myocardial infarction border area of rats among groups ($\bar{x} \pm S$)

Group	n	VEGF mRNA
Sham operation	8	50.93±7.19*
Model	8	65.41±5.93$^{\#}$
Shexiangbaoxin Pill	8	91.30±7.98$^{*\#}$
Xuesetong Capsule	8	90.00±8.53$^{*\#}$

Notes: $^{*}P$<0.05 vs model group, $^{\#}P$<0.05 vs sham operation group.

DISCUSSION

Coronary heart disease (CHD) is a major disease that severely harms human health and can result in death. CHD treatments include medication, interventional therapy and surgery. These treatments show beneficial therapeutic effects, but with shortcomings. Thus, searching for other solutions for blood circulation restoration to relieve symptoms of CHD, improve myocardial ischemia and preserve the myocardium are current research focuses. Inducing angiogenesis and promoting formation of collateral circulation are a major research focus in the international CHD research field in recent years, which may replace interventional therapy and coronary bypass.

MVD is an effective and commonly used index, which reflects angiogenesis intensity. Presently, CD34 is a more specific and sensitive surface marker of vascular endotheliocytes, with high specificity and stronger reproducibility[4], compared with those of other markers. Therefore, in this study, an anti-CD34 monoclonal antibody was used to detect CD34 expression in vascular endotheliocytes to reflect angiogenesis states, as well as show and calculate MVC and MVD. Molecular biology studies indicate that at the myocardial anoxia, angiogenesis is induced by the action of growth factors[7], particularly VEGF and basic fibroblast growth factor (bFGF) [8]. In addition, VEGF is a regulatory factor with the highest specificity and strongest function in promotion of angiogenesis[9-11]. The VEGF level can reflect ischemic degrees of the myocardium and is closely associated with lesional degrees of the coronary artery[12]. Therefore, an in situ hybridization method was used to detect the effect of Xuesetong Soft Capsules on local VEGF mRNA expression in ischemic myocardium after myocardial infarction in rats to investigate the mechanism of promoting angiogenesis at the genetic level. Previous reports indicate that Shexiangbaoxin Pills promote angiogenesis and the expression of VEGF and bFGF in ischemic myocardium after myocardial infarction in rats[1,13], and thus was used for the control.

In this study, it was shown that MVC and MVD as well as VEGF mRNA expression in the model group were higher than those in the sham operation group, indicating that ischemia and hypoxia induce angiogenesis. Xuesetong Soft Capsules significantly increased MVC and MVD in the myocardial infarction border area after myocardial infarction, which was similar to that of Shexiangbaoxin Pills, suggesting that Xuesetong Soft Capsules promote or induce angiogenesis in ischemic myocardium after myocardial infarction in rats. In addition, it was found that after administration of Xuesetong Soft Capsules, VEGF mRNA expression was significantly increased in ischemic myocardium, with a similar action of Shexiangbaoxin Pills and the same trend of increased MVC and MVD, indicating that the angiogenesis-promoting action of Xuesetong Soft Capsules and Shexiangbaoxin Pills is associated with VEGF mRNA expression.

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